IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Case Docket No. 8141

In the Application of
Inventor(s): MATTHEW JOSEPH DOYLE, ET AL.
Serial No.: 09/607,602 Group Art Unit: 1644
Date Filed: June 30, 2000 Examiner: A. DeCloux
Title: PROMOTING WHOLE BODY HEALTH

DECLARATION UNDER 37 CFR § 1.132

The Commissioner For Patents
Washington D.C. 20231

Dear Sir:

I, Robert Ernest Singer, Jr., hereby declare the following:

1) I am a co-inventor in the above-identified application.

2 I received a B.S. degree in Zoology in 1969 from Northern Illinois University and a Ph.D. in Molecular Biology/Biochemistry in 1973 from the University of Iowa.

3) Since 1973 I have been employed at The Procter & Gamble Company, where I am currently a Research Fellow in the Oral Care Technology Development Department. Since 1973 I have been conducting research into the etiology and control of periodontal disease. I have led a wide variety of basic and applied research programs focused on understanding the microbial and biochemical factors contributing to the disease process, and identifying host mechanisms of disease susceptibility and resistance. I have been involved in developing and applying diverse *in vitro*, pre-clinical *in vivo*, and clinical epidemiology models to build a better understanding of the periodontal disease process. For example, in the 1970's we first demonstrated that short chain fatty acids from anaerobic bacteria are important etiologic factors, and in the 1990's we demonstrated the important role of gingival crevicular fluid IgA as a marker for host resistance to periodontitis. This basic research has led to pre-clinical and clinical investigations into the efficacy and mechanism of action of a large number of therapeutic, preventative and diagnostic agents. More recently my research has included investigations into the relationship of periodontal disease and related mechanisms to systemic disease. Together, this work has resulted in a number of presentations at international meetings, publications, and led to the marketing of the highly effective anti-gingivitis mouthrinse, Peridex.

4) Based on our learning in this area as well as our testing of the topical H2 antagonists, I proposed and patented the use of topical formulations containing H2 antagonists to provide significant

benefits versus the prevention and control of periodontal disease (US 5,294,433, issued March 15, 1994 and US 5,364,616, issued November 15, 1994 to Singer and Ebel).

5) Subsequent to the issuance of these patents, Page, et al. reported in *Periodontology* 2000 14:9-11 (June 1997) that the host response to periodontal pathogens can be represented as a barrier function composed of the complementary interaction of gingival crevicular polymorphonuclear leukocytes (PMN's) and a local antibody response. This barrier function serves to prevent the diffusion of pathogenic substances and bacteria into the gingival tissues. Since the presence of pathogen-specific antibodies markedly enhances (~100X) the ability of PMN leukocytes to phagocytize and kill bacterial pathogens, these two mechanisms work in a synergistic fashion to maintain the barrier function. In turn, maintaining the function of both of these mechanisms is key to protection from periodontal disease. Disrupting either local specific antibody production or gingival crevicular PMN function predisposes to and is correlated with an increased risk of developing periodontal disease.

6) Subsequent to the aforementioned Singer and Ebel H2 antagonist patents, a series of papers was published that demonstrates periodontal disease is a correlate and putative risk factor for various systemic diseases/disorders such as atherosclerosis [Beck et al., *J. Periodontology*, 67, 1123-1137 (1996)], stroke [Wu et al., *Arch. Intern. Med.*, 160, 2749-55 (2000)], diabetes [Grossi et al. *Ann Periodontol.*, 51-61 (1998)], and low birth weight infants [Offenbacher et al., *J. Dent Educ.*, 62, 852-58 (1998)]. Development of periodontal disease is known to be accompanied by the diffusion of periodontal bacteria and/or their toxins into the gingival tissue and the blood stream. The hypothesized mechanism by which periodontal disease impacts on systemic diseases is that exposure of periodontal pathogens (and/or their toxic byproducts) to the systemic circulation directly and/or indirectly activates inflammatory processes that contribute to the development of atherosclerosis and the other systemic diseases. Thus, we published a paper [Ebersole et al., *J. Periodontol Res.*, 34, 358-62 (1999)] in which we showed in a pre-clinical primate model that induction of experimental periodontal disease induced an increase in serum levels of key acute phase reactant bio-markers and risk factors for atherosclerosis. Several investigators have reported [Chiu, B., *Am Heart J.*, 138, 5534-6 (1999)] that periodontal pathogens can be detected in atheromas, indicating they can lodge in the target cardiovascular tissues.

7) Subsequent to the aforementioned Singer and Ebel H2 antagonist patents, under my direction or control, a series of studies was conducted to better understand the mechanism of action of the H2 antagonists. As a result of these studies, we unexpectedly discovered that topical treatment of the oral tissues with H2 antagonists serves to increase the gingival barrier function of the periodontal tissues. Thus, in clinical models we demonstrated that topical H2 antagonists could increase gingival crevicular PMN function for the phagocytosis and killing of bacterial pathogens. [See Table 1 below and Attachment I Van Dyke et al., Int'l Assoc. for Dental Res. Annual Meeting, March 2002, Abstract # 4073, attached.]

Table 1: Clinical Efficacy for Topical H2 Antagonist for Increasing Bacterial Kill by Subgingival PMN's

Variable	Placebo	Cimetidine	% Control	p=
Number of phagocytosing PMN's/subject	13.7	31.1	227	0.03
Per cent dead bacteria/phagocytosing PMN	46.2%	63.4%	137	0.07
Number of bacteria phagocytosed	120.3	218.11	181	0.11

Following a regimen of intensive oral hygiene, twenty adult subjects enrolled in an experimental gingivitis (EG) clinical trial entailing 28 days of no toothbrushing. During EG subjects (10 subjects/group) rinsed twice daily with either a placebo mouthrinse or a mouthrinse containing 0.5% Cimetidine. On day 28, neutrophils were harvested from pre-specified gingival sulcular sites, purified, stained and examined in the trifluorochrome phagocytosis and killing microassay.

We also found in a pre-clinical model that H2 antagonists elevated the levels of gingival crevicular antibodies during experimental periodontitis as shown in Tables 2 and 3. [See also Attachment II Ebersole et al., IADR Annual Meeting, March 2002, Abstract # 4007.]

Table 2. Onset of Primate Exptl. Periodontitis: Cimetidine Efficacy v. Clinical Indices

Clinical Indices	Rank of Indices	P=
Bleeding Index	Placebo>Cim	0.0003
Pocket Depth	Placebo>Cim	0.0013
Attachment Loss	Placebo>Cim	0.0025

Following repeated dental prophylaxes to achieve gingival health at baseline, primates were entered into a double-blind study wherein the experimental group (n=15) received daily s.c. injection of 50mg/kg of Cimetidine and the control group (n=22) received injection with a placebo carrier fluid. At baseline, all subjects were placed on a soft chow diet for one month to induce gingivitis at which time ligatures were applied to study teeth to induce experimental periodontitis. Clinical indices were assessed at the one-month time point.

Table 3. Onset of Primate Exptl. Periodontitis: Cimetidine Effects on GCF Immunoglobulin Concentrations

Ag-Specific and Total GCF Ig's	Rank of Mean Concentrations	P=
IgG-Aa	Cim>Placebo	0.016
IgG-Pi	Cim>Placebo	0.0093
IgA total	Cim>Placebo	0.0012
IgG total	Cim>Placebo	0.042

Following one month of experimental peridontitis, gingival crevicular fluid samples were collected and concentrations of the respective antigen-specific IgG species (Aa = *A. actinomycetemcomitans*; Pi = *P. intermedia*) and total IgG and IgG determined with ELISA.

In other unpublished clinical research, we have found that topical application of an H2 antagonist significantly increases the levels of gingival crevicular fluid (GCF) IgA, a marker for the protective gingival tissue response as summarized in Table 4. Taken together, these studies indicate that H2 antagonists enhance the function of key mechanisms of the gingival barrier function.

Table 4. Effects of Cimetidine Mouthrinse on GCF Concentrations of IgA in a Clinical Population

GCF Variable	Mean % Increase v. Placebo	P=
IgA	86	0.03

One hundred fourteen adult subjects were given dental prophylaxis and allocated (57/group) to groups receiving either a placebo or 0.2% cimetidine mouthrinse. Following four months of supervised mouthrinse use (2x/day), GCF samples were collected and analyzed for levels of total IgA, an immunoglobulin whose concentrations in GCF have been associated with increased resistance to development of periodontitis. After adjusting for the respective baseline GCF IgA concentrations, the mean four-month GCF IgA levels were calculated for both groups and compared.

8) Based on these new findings, it is now evident that the topical application of H2 antagonists to oral tissues represents a unique and unanticipated approach to increasing the barrier function of periodontal tissues. The ability of H2 antagonists to increase the natural barrier function of gingival tissues is an extremely important benefit inasmuch as this unique mechanism of action enables providing not only a benefit versus periodontal disease but unexpectedly also represents an effective approach to preventing oral pathogens and their products from entering into either the gingival tissues or the systemic circulation. Consequently, topical application of H2 antagonists affords unanticipated benefits for preventing oral pathogens from prompting the systemic inflammatory mechanisms and complications that contribute to systemic diseases/disorders such as atherosclerosis, stroke, diabetes, and low birth weight infants.

The undersigned declares that all statements made herein which are of declarant's own knowledge are true and that all statements made on information and belief are believed to be true. I understand that the making of willful false statements and the like in this declaration is punishable by fine or imprisonment, or both, under 18 USC § 1001, and jeopardizes the validity of this application or any patent issuing therefrom.

Further Declarant sayeth naught.

Robert Ernest Singer, Jr.

Date: 3/14/02

18 USC 1001. Whoever, in any matter within the jurisdiction or any department or agency of the United States knowingly and willfully falsifies, conceals or covers up by any trick, scheme, or device a material fact, or makes any false, fictitious or fraudulent statements or representations, or makes or uses any false writing or document knowing the same to contain any false, fictitious or fraudulent statement or entry, shall be fined not more than $10,000 or imprisoned not more than five years, or both.

Attachment I

4073 Effects of topical cimetidine rinse on gingival crevicular neutrophil leukocyte function

T.E. VAN DYKE[1], C.W. CUTLER[2], M. KOWOLIK[3], R.E. SINGER[4], W. BUCHANAN[4], and **A.R. BIESBROCK**[4], [1] Boston University School of Dental Medicine, USA, [2] SUNY at Stony Brook, USA, [3] Indiana University, USA, [4] The Procter and Gamble Company, USA

Objectives: Three pilot clinical studies were conducted to examine the effects of topical cimetidine rinse on neutrophil function in the gingival crevice. **Methods:** The first study was a randomized, double blind, placebo controlled, 28 day experimental gingivitis study involving 21 healthy adults in which subjects rinsed twice a day with placebo or 0.5% cimetidine rinses. At baseline, day 14, 21, and 28, neutrophils were harvested from pre-specified gingival sulcular sites, purified, stained and examined in the trifluorochrome phagocytosis and killing microassay. The second and third studies were placebo controlled, 9 week, three period, longitudinal studies involving 6 and 9 adults with moderate periodontitis, respectively. Subjects rinsed twice a day with during periods 1 and 3 with placebo and during period 2 with 0.5% cimetidine. At baseline and weekly intervals, neutrophils were harvested from pre-specified periodontal pockets, purified, stained and examined in the trifluorochrome phagocytosis and killing microassay in the second study. In the third study, neutrophils were examined spectrophotometrically for superoxide production and in a luminol-enhanced chemiluminescence assay. LS means for each group were examined by two sided student t-test **Results:** In the first study, the mean number of phagocytosing neutrophils were significantly increased ($p=0.016$) in the cimetidine group (31.1 cells/subject) versus the placebo group (13.7 cells/subject) at day 28. In addition, a significant increase ($p=0.036$) in bacterial killing was observed in the cimetidine rinse group. In the cimetidine group, 63.4% of bacteria in the neutrophils were killed relative to 46.2% in the placebo group. Data from the other two studies in periodontal subjects provide supporting evidence of enhanced neutrophil function in conjunction with topical cimetidine therapy. **Conclusions:** Collectively, these pilot studies provide evidence that topical 0.5% cimetidine oral rinse enhances the anti-bacterial function of crevicular neutrophils. Supported by The Procter & Gamble Company.

Attachment II

4007 Effects of an H2-Receptor Antagonist on Gingivitis & Periodontitis in Nonhuman Primates (Nhp)

J.L. EBERSOLE, University of Kentucky, USA, D. CAPPELLI, UTHSCSA, USA, S.C. HOLT, The Forsyth Institute, USA, H.M. PICKRUM, The Procter and Gamble Company, USA, and R.E. SINGER, The Procter & Gamble Co, USA

Numerous studies have identified mast cells in the inflamed tissues of the periodontium. Accumulation and triggering of these cells within a chronic inflammatory lesion may result in histamine release. Histamine modulates immune responses via H_2-receptors on inflammatory and immune cells. **Objective:** An hypothesis regarding the progression of periodontitis is a localized inflammatory/immune dysregulation that results from the chronic qualitative/quantitative stimuli by the biofilm. These studies were designed to evaluate the capability of an H_2-receptor antagonist (cimetidine; CM) to alter the microbiota, inflammatory/immune response, and clinical presentation of periodontitis in Nhp. **Method:** Experiment 1 was a double-blind study: experimental group (2CM; n=10) daily s.c. injection of 50mg/kg of CM; control group (2P; n=10) injected with a placebo carrier fluid. Experiment 2 was a double-blind study: experimental group (4CM; n=15) daily s.c. injection of 50mg/kg of CM and control group (4P; n=22) injected with a placebo carrier fluid. Clinical and biological samples were collected at baseline, through 4 weeks of experimental gingivitis, and through 3 months following ligature-induced periodontitis. **Result:** Exp.1 demonstrated that the 2CM had lower attachment loss (AL), bleeding (BOP), probing depths (PD), and recession than 2P. CADIA determination of bone loss demonstrated a significant decrease (p=0.021) in the 2CM. Host responses suggested an increased total IgG, IgA and PGE_2 in GCF during ligation in the 2CM. Exp. 2 showed the 4CM with less AL, PD, BOP, and plaque than 4P. The 4CM also demonstrated elevated IgG, IgA, and antibody in GCF. Significant positive correlations between antibody levels and clinical parameters were only noted in the 4CM group. Significant positive correlations between changes in the microorganisms and antibody levels were observed, although minimal microbiological differences were noted between the groups. **Conclusion:** These results support that **CM modulated host responses associated with decreased disease, potentially via improved regulation of the local responses**. Supported by Procter & Gamble.